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Enzymatic-modified dietary fibre fraction extracted from potato residue regulates the gut microbiotas and production of short-chain fatty acids of C57BL/6 mice

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ABSTRACT

Potato residue is a rich source of dietary fibre. Herein, the effects of unmodified and enzymes (cellulase and xylanase) modified potato residue dietary fibres on distribution of intestinal microbiotas and production of short-chain fatty acids (SCFAs) of C57BL/6 mice were evaluated. Results showed that enzymatic modification increased soluble dietary fibre content in potato residue. Administration of these dietary fibre promoted the production of acetic acid, n-butyric acid, iso-butyric acid, valeric acid, and iso-valeric acid, while inhibiting propionic acid content ($p < 0.05$). The potato residue dietary fibre improved the richness and diversity of intestinal microflora of mice, particularly increased *Bacteroidetes* to *Firmicutes* ratio ($p < 0.05$). Modification by cellulase and xylanase significantly improved the regulating bioactivities towards gut microbiota and increased values in food applications of potato residue dietary fibres. These dietary fibres have the potential to act as therapeutic agents to treat dysbiosis in the intestinal microbiota.

1. Introduction

Recent studies have suggested that the gastrointestinal microbiota in human highly participates in yielding, storing, and expending energy from the diet, which may be a further factor in linking diet with metabolic syndrome (Davis, 2016). The appropriate consumption of dietary fibre has been shown to reduce blood sugar, lower fat, and prevent cancer development (Chen et al., 2016). The composition of gut microbial communities is greatly affected by dietary habit. It is dynamic and efficient in response to the dietary alterations, in particular increasing or decreasing the dietary fibre intake (Leeming, Johnson, Spector, & Le Roy, 2019). Potato (*Solanum tuberosum L.*) is a widely consumed root vegetable, ranking the fourth among the food crops in the world (Yang et al., 2020). It has a high nutritional content of starch, protein, dietary fibre, vitamins, and minerals. Nowadays, potato starch has been rapidly applied in the food industry as it has a longer preservation time compared to other potato food products. Production of one ton of potato starch can produce 0.2–0.5 tons of by-products, including potato peel and residue (Birch et al., 2012). These by-products are rich sources of dietary fibre with high moisture content and are not easy to be stored and transported. Therefore, proper treatment and rational reuse should be carried out to enhance their waste values.

Dietary fibre can be divided into water-soluble dietary fibre (SDF) and water- insoluble dietary fibre (IDF). The digestion and decomposition of dietary fibre are mainly carried out in the large intestine, and the intestinal microorganisms, including bacteria, archaea, and fungi, in the large intestine will selectively decompose, ferment and utilize the dietary fibre (Barber, Kabisch, Pfeiffer, & Weickert, 2020). These intestinal microorganisms, especially gut bacteria can secrete different enzymes in the process of reproduction and metabolism to hydrolyse the dietary fibre, in particular the SDF, consequently becoming the nutrients

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for the growth of intestinal bacteria. These products utilized by intestinal bacteria in turn affect the type and quantity of intestinal flora (Holscher, 2017). Studies (Jha, Fouhse, Tiwari, Li, & Willing, 2019; Myhrstad, Tunsjø, Charnock, & Telle-Hansen, 2020) have demonstrated that dietary fibre can be used as an energy source and nutrient for aerophilic microorganisms to promote the growth and reproduction of aerophilic microorganisms. In addition, intestinal microorganisms use dietary fibre to produce a large number of short-chain fatty acids (SCFAs), such as acitic acid, propionic acid and butyric acid by fermentation, which in turn, inhibit the growth and reproduction of pathogenic bacteria and carcinogens, and prevent intestinal diseases (Markowiak-Kopeć & Śliżewska, 2020). Therefore, it is of great importance to explore the fermentability of potato residue dietary fibre in the intestinal tract and its influence on the intestinal microflora for the future applications of potato residue dietary fibre in food industries.

SDF plays a conducive physiological function compared to IDF. SDF exhibited not only a conducive physiological function, but also a stronger capacity to provide viscosity and form gels compared to IDF. Capuano (2017) evaluated the effect of the added dietary fibre on the gastrointestinal fate of emulsified lipids by simulating the gastrointestinal tract. Their findings suggested that the rate and extent of lipid digestion can be controlled by the addition of the dietary fibre, which could be a useful information for developing functional foods. McRae (2018) also recommended that an average daily dose of 18 g/day of SDF could effectively reduce the fasting blood glucose. However, the SDF content extracted from natural plants is too low to exert its physiological functions in human body (Archacka et al., 2020). Hence, it is also necessary to investigate a novel modification technique to improve the quality of dietary fibre by elevating SDF content.

In this study, the effects of unmodified and enzymatic-modified potato residue dietary fibres, expressed as UTDF and ETDF, respectively, on the distribution of intestinal microbiotas and the production of SCFAs were evaluated and compared via gastric administration of these dietary fibres for C57BL/6 mice. The findings of this study may provide a theoretical basis for increasing the utilization of the by-products produced from the potato powder processing, and also for the future application of dietary fibre in food industries.

2. Materials and methods

2.1. Chemicals and materials

The standards for determining the monosaccharide composition included mannose, ribose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, xylose, arabinose, and fucose, which were purchased from the Sigma-Aldrich Co. (St. Louis, MO, USA). Cellulase and xylanases were purchased from Solarbio (Beijing, China). Potato residuals were kindly provided by Zhangjiakou Hongji Agricultural Technology Development Co., Ltd. (Hebei, China). Other reagents were all of analytical grade.

2.2. Pre-treatment of potato residue

The by-product from potato starch (including the potato residue) was freeze dried in a vacuum freeze drier for 48 h. The lyophilized potato residue was crushed, and then sieved through a 40-mesh sieve. Samples were stored in a vacuum desiccator in the dark for subsequent analysis.

2.3. Extraction and modification of dietary fibre extracted from potato residue

The extraction and modification technology for dietary fibre extracted from potato residue was optimized by single factor tests and response surface analysis in our preliminary study. The conditions for extraction and modification of SDF and IDF extracted from potato residue has shown in Fig. 1. The IDF fraction extracted from the potato residue was modified by cellulase and xylanase hydrolysis, and the optimized modification was under the following condition: the concentration of cellulase and xylanase enzymes was 0.18%, enzymatic hydrolysis temperature was 51 °C, pH value of enzymatic hydrolysis was 4.9, and the enzymatic hydrolysis time was 2 h. The content of extracted potato residue SDF fraction under this condition reached to 26.82%.

2.4. Determination of monosaccharide composition in potato residue dietary fibre

The monosaccharide compositions in UTDF and ETDF samples were

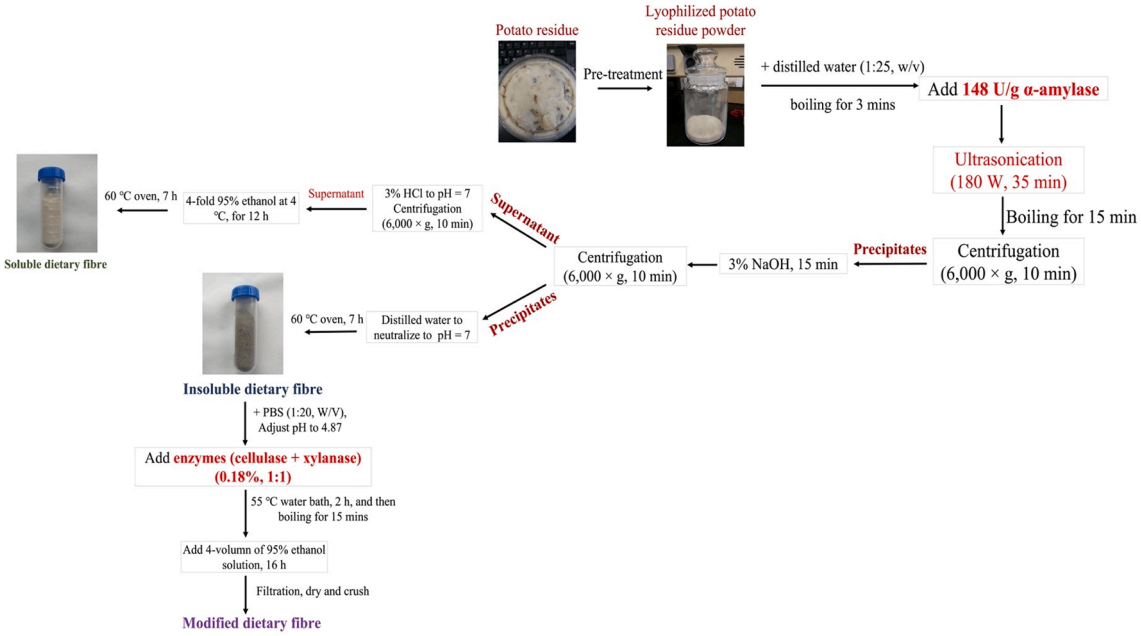

Fig. 1. . The process of extraction of soluble dietary fibre and insoluble dietary fibre from dried potato residue powder, and enzymatic modification of insoluble dietary fibre in potato residue.

both determined using an Agilent 1200 HPLC system (Pérez-López, Mateos-Aparicio, & Rupérez, 2017). Samples were hydrolysed with 4 mol/L trifluoroacetic acid at a 120.0 °C oven for 2 h, and then dried with nitrogen. The dried samples were mixed with 1 mL of 0.5 mol/L 1-phenyl-3-methyl-5-pyrazolone-methanol and 0.5 mL of 0.3 mol/L NaOH at a 70.0 °C water bath for 60 min, and then cooled to the room temperature. After adding 0.5 mL of 0.3 mol/L HCl and 0.5 mL of chloroform, the mixture was vortexed well and precipitated for 20 min. The lower layer was discarded. The extraction procedure was repeated for three times. The upper layer was pooled, and then filtered through a 0.22-μm filter for subsequent analysis. After injecting 10 μL of sample, separation was performed in a SHISEIDO® C18 column (4.6 mm × 250 mm, 5 μm) with a flow rate of 1 mL/min at 25.0 °C. The ratio of 0.1 mol/L KH_2PO_4 (pH = 6.8) to acetonitrile was 82:18 (v/v). The chromatograms were recorded at 245 nm. Glucose (Glc), galactose (Gal), galacturonic acid (GalA), rhamnose (Rha), and arabinose (Ara) were detected using their reference standards and compared to the internal standard and blank samples.

2.5. Animal experimental design

Fifty-six male C57BL/6 mice (20 ± 2.0 g) were purchased from Jinan Pengyue Experimental Animal Breeding Co., Ltd (Jinan, Shandong, China). All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Hebei Agricultural University and approved by the Animal Ethics Committee of Hebei Agricultural University (approval no. 1107261911005113). The mice were housed in stainless steel cages in a temperature-controlled room maintained on a 12 h light/12 h dark cycle. Each group was fed with the indicated diet ad libitum and allowed free access to water. After acclimation with a standard diet for one week, mice were fed with standard diet. The unmodified dietary fibre consists of 83.34% IDF and 15.16% SDF, while the enzymatic-modified dietary fibre contains 72.26% IDF and 26.82% SDF. The dietary fibre was dissolved in distilled water. Mice were divided into 7 groups (n = 8 for each group), and then intragastric administrated with 20 mL/(kg d) of low (0.25 mg/(g d), medium (0.50 mg/(g d)), and high dose (1.00 mg/(g d)) of unmodified (UTDF-L, UTDF-M, and UTDF-H, respectively), or modified potato residue dietary fibre (ETDF-L, ETDF-M, and ETDF-H, respectively). The control group (C) was administrated with 20 mL/(kg d) of distilled water. Mice faeces (at least 10 pills of faeces for each group) were collected in sterile tubes individually at day 0, 7, and 14 d after indicated administration. Immediately after the collection, samples were stored at −80 °C until SCFA and microbiota analysis. At the end of the experiment, animals were anesthetized by diethyl ether.

2.6. DNA extraction, PCR amplification and illumina MiSeq sequencing

The DNA of the faecal bacterial from mice was extracted using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The final DNA concentration and purity were determined using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, MA, USA). DNA quality was evaluated by 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5′- ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by thermocycler PCR system (ABI GeneAmp® 9700, Waltham, MA, USA). 16S rRNA gene sequencing was performed to evaluate the effects of enzymes-modified dietary fibre on the gut microbiota. A total of 1244,364 raw reads with an average of 38,770 ± 10,394 sequences per sample were generated in 15 samples. After removing the low-quality sequences, 622,182 valid sequences with an average length of 421 bp were obtained. A total of 662 OTUs were obtained to be identified 10 bacterial phyla and 146 bacterial genera. The PCR reaction was conducted using the following program: 3 mins at 95 °C, 27 cycles for 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s at 72 °C, a final extension at 72 °C for 10 min, and 10 °C until halted by user. PCR reactions were performed in triplicate: 20 μL of mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of forward and reserve primer (5 μM), 0.4 μL of FastPfu Polymerase, 0.2 μL of BSA, and 10 ng of template DNA. The resulted PCR products were extracted from a 1% agarose gel, and then purified using a AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and further quantified using QuantiFluorTM-ST (Promega, Madison, WI, USA). Afterwards, the purified amplicons were pooled in equimolar, and paired end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) by following the standard protocols of Majorbio Bio-Pharm Technology Co. Ltd.

2.7. Processing of sequencing data

Raw fastq files were quality-filtered by Trimmomatic and merged by FLASH with the following standard: The reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window; the overlap longer than 10 bp of sequences were merged according to their overlap with mismatch no more than 2 bp; Sequences of each sample were separated according to the barcodes (matching exactly) and Primers (two nucleotides mismatched were allowed). The reads containing ambiguous bases were removed. Operational taxonomic units (OTUs) were clustered with 97% similarity cut-off using UPARSE (version 7.1 http://drive5.com/uparse/). The taxonomy of each 16S rRNA gene sequence was analysed by RDP Classifier (version 2.11, https://sourceforge.net/projects/rdp-classifier/) against SILVA (version 132, https://www.arb-silva.de/) 16S rRNA database. The confidence threshold was 70%.

2.8. Quantification of SCFA content in the fermented faecal samples

SCFAs content in faecal samples were determined according to a previously study (Tao et al., 2016). 0.2 g of each sample was added into 2 mL of solution (1:3 phosphoric acid aqueous solution), and then homogenized for 2 min. 2 mL of ethyl ether was added to mix well for 10 min. The mixture was then centrifuged at 4000 r/min for 20 min (treated at low temperature and placed in an ice water bath for centrifugation). After centrifugation, the ether phase was taken out, and 2 mL of ether was added for extraction for 10 min, and centrifugation for separation at 4000 r/min for 20 min. Afterwards, the ether phase was taken out again, and the two extracts were combined to volatilize to 2 mL. The solution was filtered with a 0.22-μm filter for the ion chromatography system. A Perkin Elmer Clarus® 680 gas chromatograph (PerkinElmer, Inc., Waltham, MA, USA) equipped with a flame ionization detector (FID) was used to analyse the samples. Separation was achieved using a HP-INNOWAX column, 25 m × 0.20 mm × 0.40 mm (Agilent Technologies Inc., Santa Clara, CA, USA). The instrument detection parameters are shown in Table 1. **Figure S1** shows the gas chromatographic diagram of the mixed standard solution of six types of SCFAs, namely acetic acid, n-butyric acid, *iso*-valeric acid, propionic

Table 1
Instrument detection parameters for identification of SCFAs.

Chromatographic column	HP-INNOWAX (25 m × 0.20 mm × 0.40 μm)
Column temperature	Initial temperature at 100 ℃ for 5 mins, increased to 150 ℃ at a rate of 5 ℃/min, increased to 240 ℃ at a rate of 30 ℃/min, and then kept it for 30 mins
Inlet temperature	240 ℃
Flowrate of carrier gas	1.0 mL/min
Split ratio	no
Ion source temperature	200 ℃
Transmission line temperature	250 ℃
EI source bombardment voltage	70 eV
Single ion scanning mode	Quantitative 60, 73

acid, n-valeric acid, and *iso*-butyric acid according to their peak time. The individual SCFA content (mg/kg) was calculated according to the following formula:

$$W(mg/kg) = \frac{C*V*N}{m}$$

where C refers to the concentration of SCFA in sample solution (mg/L); V represents the constant volume (mL); N depicts the dilution factor, while M represents the weight of the sample (g).

2.9. Statistical analysis

All the determinations were conducted in triplicate unless otherwise stated. One-way analysis of variance (ANOVA) was employed to do statistical analysis, and a significant difference was obtained by Tukey's comparison test ($p < 0.05$). Pearson's correlations were also recorded to investigate all observed values. GraphPad Prism software (Version 8.0, GraphPad Software, Inc., San Diego, CA, USA) was used for data analysis.

3. Results and discussion

3.1. Monosaccharide composition in dietary fibre extracted from potato residues

HPLC was used to qualitatively and quantitatively determine the composition of monosaccharides. As shown in Fig. 2, the monosaccharide composition of the dietary fibre before and after modification were both composed of nine monosaccharides, namely mannose, ribose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, xylose and arabinose. The dominant monosaccharides before the modification were galactose (42.63% ± 1.23) and glucose (25.96% ± 0.85), while in modified dietary fibre, the content of galactose and glucose was 44.63% ± 0.93 and 20.09% ± 0.76 of total sugar, respectively. The content of mannose, rhamnose, glucuronic acid, galacturonic acid, galactose, xylose, and arabinose in modified dietary fibre was higher than those in unmodified dietary fibre ($p < 0.05$). As previous studies (Huang & Ma, 2019; Neela & Fanta, 2019) have reported that the main monosaccharides of SDF are glucose, arabinose and galactose. Therefore, in this study, the increased arabinose and galactose concentration in modified dietary fibre could be the main contribution to the increased SDF content in potato residue after the enzymes' modification. This increased SDF content further affected the water solubility of potato residue. In addition, the main component of the increased monosaccharide is hemicellulose. Cellulose degrades into hemicellulose with the modification of cellulase (Horn, Vaaje-Kolstad, Westereng, & Eijsink, 2012). Owing to the hydrophilic ability, it is easier for hemicellulose to be decomposed by bacteria and bind to ions, thus improving the group function. The hemicellulose is further hydrolysed to hexose and pentose (López-Mondéjar, Zühlke, Becher, Riedel, & Baldrian, 2016). Therefore, it can be concluded that the main components of the dietary fibre in potato residue is cellulose and part of hemicellulose. The enzymatic modification can further hydrolyse these celluloses and hemicellulose, consequently leading to the increased SDF content in potato residue.

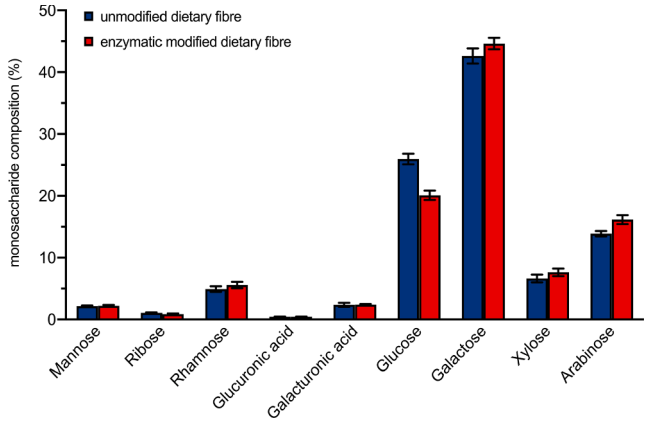

Fig. 2. . The monosaccharide compositions in the dietary fibre extracted from potato residue.

3.2. Microbial α-diversity in different groups

Table 2 shows the statistics of α-diversity index of intestinal flora of mice. Alpha-diversity analysis reflects the richness and diversity of the microbial community (Willis, 2019). Herein, Sobs, Chao, Shannon, Simpson, Ace, and Coverage index were calculated in microbial diversity analysis within the community. Sobs and Ace represent the actual observed value of richness, while the Chao is used to estimate the OTU numbers in samples (Hughes, Hellmann, Ricketts, & Bohannan, 2001). Both Simpson and Shannon depict the microbial diversity in samples. After administration with dietary fibre for 14 d, the OTUs, Sobs, Chao, Shannon, and Ace index in unmodified total dietary fibre (UTDF) (14 d) and enzymatic-modified total dietary fibre (ETDF) (14 d) groups were higher than that in the control (0 and 14 d) groups of particular the groups administrated with medium and high dose of ETDF ($p < 0.05$). This result illustrates that dietary fibre could improve the intestinal flora richness and diversity in mice at 97% identity. Both the gut flora richness and diversity may contribute human obesity and some other chronic diseases (Barczynska, Jurgoński, Slizewska, Juśkiewicz, & Kapusniak, 2017). In addition, the high dose modified dietary fibre exhibited the most effective improvement among all dietary fibre administration groups. Coverage represents the coverage of each sample library. Herein, Coverage values detected in this study are all greater than 0.99. This finding suggests that the sequence in the sample was highly likely to be detected, which could represent the distribution of bacterial community of samples.

3.3. Partial least squares discriminant analysis (PLS-DA) and Venn diagram

Fig. 3**A** shows the PLS-DA diagram. PLS-DA analysis is an effective method to evaluate if there is significant difference between different groups, and to find the influencing variables that lead to differences between groups (Ruiz-Perez, Guan, Madhivanan, Mathee, & Narasimhan, 2020). As shown in the figure, similarity analysis between groups was performed on samples at the OTU level. Obtained values in the same group are relatively uniform. The results show that there are obvious differences between the UTDF, ETDF, and the control groups. The great differentiation among these three groups indicates that there is statistical difference between the intestinal flora of each group. This finding reveals that administration of dietary fibre significantly affected the bacterial community structural composition in mice. However, the similarity in intestinal flora between UTDF and ETDF groups is higher than that between the control and UTDF, or control and ETDF groups. This observation is agreement with the results of the main monosaccharide compositions in dietary fibre. Unmodified- and enzymatic-modified dietary fibre had the same source as the main monosaccharide compositions in both dietary fibres were cellulose and part of hemicellulose. Therefore, the effects of enzymatic-modified dietary fibre on the structural characteristics of the intestinal flora in mice had similarities with unmodified dietary fibre. Fig. 3**B & 3C** is the Venn diagram of species. Venn diagram can be used to evaluate the number of both common and unique species in multiple samples, and visually display the similarity and overlap of species composition in different samples (Wang, Coleman-Derr, Chen, & Gu, 2015). As shown in Fig. 3**B**, the OTU number of the control group, unmodified dietary fibre group (UTDF), and enzymatic-modified dietary fibre group (ETDF) was 405, accounting

Table 2
Richness and diversity indexes from faecal samples of each group.

	OTUs	Sobs	Chao	Shannon	Simpson	Ace	Coverage
0 d	521.33 ± 4.16^{f}	373.33 ± 8.62^{d}	448.07 ± 37.65^{a}	3.73 ± 0.02^{e}	0.07 ± 0.00^{c}	444.85 ± 28.51^{ab}	0.9976 ± 0.0002^{ab}
Control (7 d)	511.00 ± 2.65 ^{g}	368.00 ± 6.56d^{e}	427.37 ± 33.98^{a}	3.74 ± 0.02^{e}	0.07 ± 0.02^{c}	426.14 ± 26.75^{b}	0.9973 ± 0.0002^{b}
UTDF-L (7 d)	534.33 ± 13.58^{ef}	422.67 ± 9.71^{b}	512.82 ± 41.67^{a}	4.34 ± 0.02^{a}	0.03 ± 0.00^{i}	518.37 ± 35.81^{a}	0.9964 ± 0.0003^{d}
UTDF-M (7 d)	520.67 ± 3.06f	374.00 ± 4.58^{d}	456.51 ± 44.65^{a}	3.02 ± 0.03 ^{h}	0.21 ± 0.00^{j}	444.68 ± 30.77^{ab}	0.9974 ± 0.0002^{b}
UTDF-H (7 d)	522.00 ± 4.58^{f}	372.33 ± 8.08^{d}	443.20 ± 40.11^{a}	4.11 ± 0.02^{d}	0.04 ± 0.00^{e}	443.02 ± 32.22^{ab}	0.9964 ± 0.0002^{d}
ETDF-L (7 d)	534.33 ± 4.51^{e}	308.00 ± 5.29^{f}	346.15 ± 23.53^{b}	3.66 ± 0.02^{f}	0.08 ± 0.00^{b}	345.39 ± 18.52^{c}	0.9982 ± 0.0003^{a}
ETDF-M (7 d)	540.33 ± 2.08^{e}	354.33 ± 8.62^{e}	469.71 ± 63.37^{a}	3.08 ± 0.02 ^{g}	0.18 ± 0.00^{c}	426.50 ± 31.77^{b}	0.9973 ± 0.0002^{bc}
ETDF-H (7 d)	546.67 ± 3.21^{d}	421.00 ± 8.54^{b}	475.62 ± 30.63^{a}	4.17 ± 0.02^{c}	0.04 ± 0.00^{e}	479.20 ± 26.18^{ab}	0.9974 ± 0.0002^{b}
Control (14 d)	541.00 ± 5.29^{de}	405.67 ± 5.13^{b}	472.18 ± 34.53^{a}	3.61 ± 0.02^{f}	0.08 ± 0.00^{ab}	479.39 ± 30.88^{ab}	0.9969 ± 0.0002^{c}
UTDF-L (14 d)	554.00 ± 6.24 ^{cd}	384.00 ± 10.54 ^{cd}	485.85 ± 54.10^{a}	4.07 ± 0.02^{d}	0.04 ± 0.00^{f}	475.60 ± 39.03^{ab}	0.9967 ± 0.0002^{cd}
UTDF-M (14 d)	576.33 ± 7.51^{bc}	392.33 ± 4.73^{c}	469.77 ± 41.90^{a}	3.72 ± 0.02^{e}	0.09 ± 0.00^{a}	461.51 ± 29.94^{ab}	0.9974 ± 0.0002^{b}
UTDF-H (14 d)	623.33 ± 5.69^{a}	430.00 ± 7.94^{ab}	491.99 ± 33.90^{a}	4.13 ± 0.02 ^{cd}	0.05 ± 0.00^{d}	482.22 ± 23.14^{ab}	0.9974 ± 0.0003^{b}
ETDF-L (14 d)	567.00 ± 7.00^{c}	371.67 ± 5.86^{d}	475.62 ± 50.58^{a}	3.03 ± 0.03 ^{h}	0.18 ± 0.00 ^{k}	474.12 ± 39.55^{ab}	0.9964 ± 0.0001^{d}
ETDF-M (14 d)	593.33 ± 8.33^{b}	443.67 ± 8.08^{a}	512.36 ± 36.49^{a}	4.30 ± 0.02^{b}	0.03 ± 0.00 ^{h}	516.08 ± 30.68^{a}	0.9976 ± 0.0002^{b}
ETDF-H (14 d)	631.00 ± 5.29^{a}	442.33 ± 9.61^{ab}	511.04 ± 36.52^{a}	4.30 ± 0.02^{b}	0.03 ± 0.00 ^{g}	509.32 ± 28.56^{a}	0.9973 ± 0.0001^{b}

Values are mean ± standard deviation (mean ± SD, n = 3). Values with different lowercases in the same column differ from each other statistically ($p < 0.05$) Abbreviations: UTDF = unmodified total dietary fibre; ETDF = enzymatic-modified total dietary fibre. L, M, and H represents the low, medium, and high dose of dietary fibre, respectively.

for around 61.18% of the total OTU number. The OTU number of UTDF and ETDF groups were both higher than that of the control group, and the OTU number of ETDF group was higher than that of UTDF group ($p < 0.05$). The number of OTU shared by UTDF and ETDF groups was 112, which was significantly higher than that of control and UTDF groups, as well as control and ETDF groups ($p < 0.05$). These findings indicate that the number of intestinal flora species were positively influenced by the dietary fibre. The intestinal flora in E group was similar to those UTDF group. Fig. 3**C** exhibits the similarity of species composition between UTDF-L, UTDF-M, and UTDF-H (low, medium, and high dose of unmodified dietary fibre, respectively) groups and ETDF-L, ETDF-M, and ETDF-H (low, medium, and high dose of enzymatic-modified dietary fibre, respectively) groups. The OTU number shared by these six groups was 297, accounting for 44.86% of the total OUT number, among which the OTU number of ETDF-M and ETDF-H group was 494 and 506, respectively, which were higher than that of UTDF-M and UTDF-H group (481 and 501, respectively). These observations suggest that the number of intestinal microbiota species in mice was correlated with the amount of dietary fibre administrated with mice. It was positively correlated with the medium and high dose dietary fibre-treated groups.

3.4. The composition of the intestinal flora in mice at phylum, family, and genus levels

Fig. 4 shows the composition of the intestinal flora in mice at the levels of phylum (A), family (B), and genus (C). According to the Fig. 4**A**, at the level of phylum, *Bacteroidetes* and *Firmicutes* are the dominant bacterial communities in the intestinal flora of mice. The contents of *Bateroidetes* and *Firmicutes* in control, UTDF, and ETDF groups accounted for the highest of all bacterial communities, followed by the content of Proteobacteria, *Deferribacteres* and *Actinobacteria*. *Epsilonbecteraeota* is also identified in this study, which is a non-pathogenic bacterium (Hewitt, Díez-Vives, & Taboada, 2020), which is widely found in a variety of natural environments around the world. *Epsilonbecteraeota* is commonly observed as the dominant species in microbial communities. As the dominant flora, *Firmicutes* and *Bacteroidetes* accounted for 15.69% and 76.48% of the intestinal flora of mice in control group, respectively. The proportion of *Firmicutes* in UTDF-L, UTDF-M and UTDF-H groups was 25.76%, 18.00%, 49.99%, respectively, whilst it was accounted for 68.49% (ETDF-L), 33.55% (ETDF-M), and 22.62% (ETDF-H) in enzymatic dietary fibre-treated group, which were all higher than that of control group. This finding indicates that administration of UTDF or ETDF was beneficial to the growth of Firmi*cutes* microorganisms. This is consistent with the report of Cui et al. (2019). In addition, the proportion of *Firmicutes* in UTDF-H and UTDF-L groups were the highest among all groups, suggesting that UTDF-H and ETDF-L had the most significant effects on the intestinal flora of mice. As the increasing dose of dietary fibre administrated with mice, the relative content of *Firmicutes* in intestinal microflora of mice decreased, while the relative content of *Bacteroidetes* increased (Fig. 4**B**). Studies (Clarke et al., 2012; Grigor'eva, 2020) have revealed that increasing the number of *Bacteroidetes* and reducing the number of *Firmicutes* could reduce the probability of obesity. Hence, administration of UTDF or ETDF could be a potential therapy for preventing obesity. This observation is also agreement with a previous study (Liu et al., 2020), which reported the effects of dietary fibre extracted from sweet potato residue on the diversity of intestinal flora. Their finding showed that dietary fibre from sweet potato residue promoted the increase the content of *Bifidobacterium* and *Lactobacillus,* and inhibited the growth and reproduction of *Bacteroidetes*, *Enterobacterium* and *Clostridium perfringens*. Collectively, administration of potato residue dietary fibre inhibited the growth of *Bacteroidetes.* This inhibitory effect of ETDF was higher than that of the UTDF.

Fig. 4**C** shows the composition of mice intestinal flora at the level of family classification. The relative richness of intestinal flora of UTDF and ETDF groups increased significantly compared with the control group, among which *Muribaculaceae, Prevotellaceac, Veillonellaceae, Bacteroidaceae, Rikenellaceae, Lachnospiraceae, and Ruminococcaceae* were the dominant flora. *Muribaculaceae* has no obvious change pattern between different groups in this study. *Prevotella* microorganism has been reported to be positively correlated with obesity and bowel diseases (Hills et al., 2019). Identified in numbers of studies (Illiano, Brambilla, & Parolini, 2020; T. Su et al., 2018), a high predominance of *Prevotella* to *Bacteroides* has been shown in contrast to the faecal microbiota of those living in Westernised societies (Archacka et al., 2020). In this study, the proportion of *Prevotellaceae* in the intestinal flora of control group was 47.75%, which was significantly higher than that of UTDF and ETDF groups. The inhibitory effect of ETDF on *Prevotellaceae* was more obvious than that of UTDF. This observation indicates that administration of dietary fibre with mice inhibited the growth of *Prevotella*, and therefore probably reducing the risk of obesity and bowel diseases. *Veillonellaceae* was widely found in UTDF-H and ETDF-L groups. The proportion in other groups was very low. In addition, treatment with ETDF-L to mice was more conducive to the proliferation of *Veillonellaceae* than that of UTDF-H. The proportion of *Bacteroidaceae* in control group was 4.27%. Administration of dietary fibre promoted the growth of *Bacteroidaceae* microorganisms. The proportion of *Bacteroidaceae* in ETDF-H group was the highest, accounting for 18.31% of total intestinal microorganisms. However, there was no significant difference between other dose groups, suggesting that the dose of dietary fibre may have no effect on the number of *Bacteroidetes*.

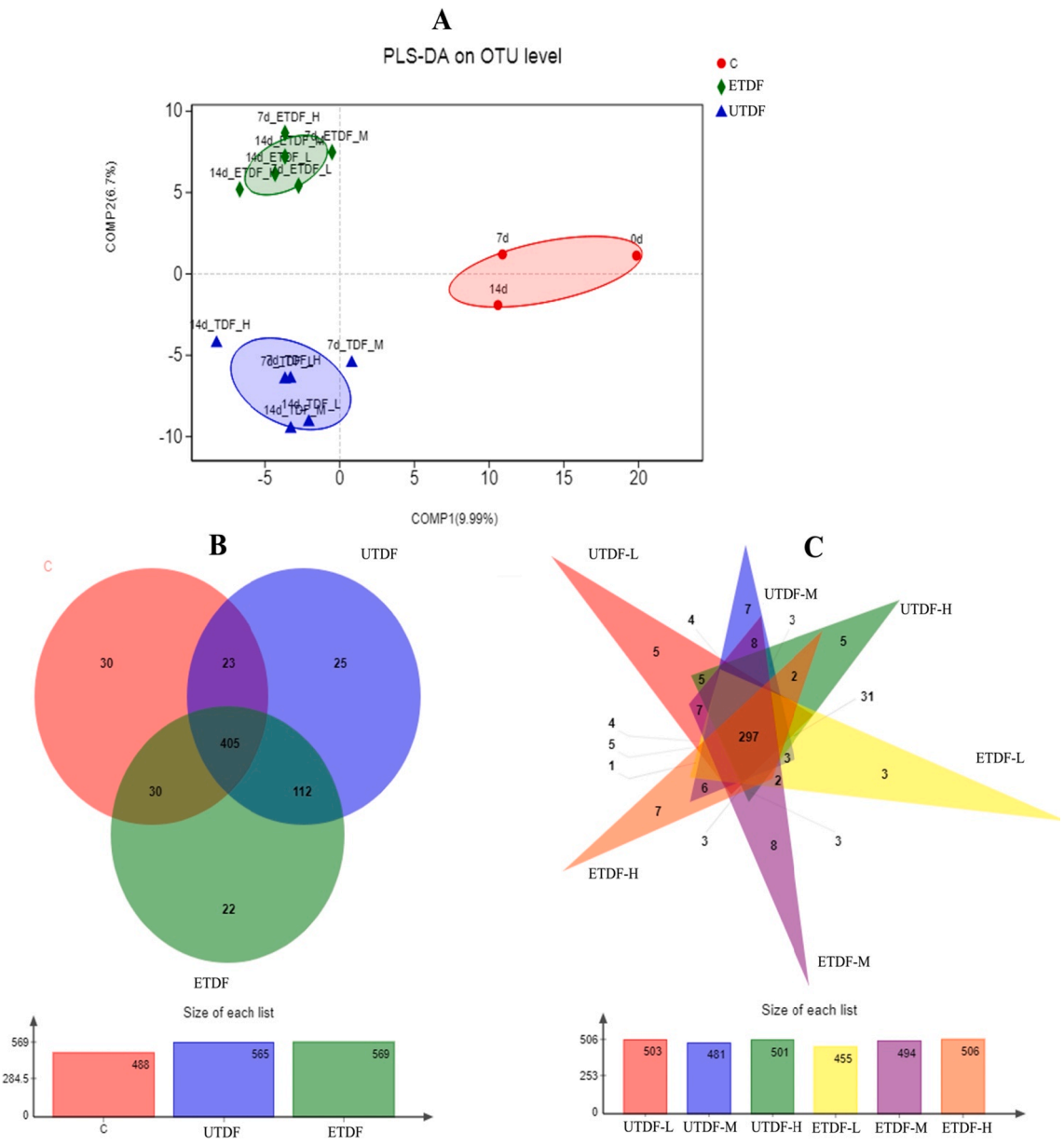

Fig. 3. . Partial Least Squares Discriminant analysis (PLS-DA) (A) and Venn diagram (B & C) for control, unmodified, and enzymatic-modified dietary fibre groups.

Except for the ETDF-L group, the proportion of *Lachnospiraceae* and *Ruminococcaceae* microorganisms in the UTDF and ETDF groups was higher than that in the control group. As a potential beneficial bacterium, *Lachnospiraceae* participates in the metabolism of carbohydrates, while *Ruminococcaceae* decomposes cellulose and pectin (Vacca et al., 2020). Herein, the microbial content of *Lachnospiraceae* and *Ruminococcaceae* increased, indicating that dietary fibre extracted from potato residue induced the growth of beneficial bacteria *in vivo*, and promoted the Ruminococcaceae to effectively decompose and digest dietary fibre. There is significant association between SCFAs and composition of the microbiota (den Besten et al., 2013). The fermentation of high luminal concentrations (in the proximal colon where fermentation is the highest, pH value is between 5.5 and 6.5, while in the distal colon, the pH value is from 6.5 to 7.0.) can lower the colonic pH value and inhibit growth of Gram-negative. Fig. 4**D** shows the composition of intestinal microflora of mice at the level of genus classification. Compared with the control group, the relative richness of intestinal flora in UTDF and ETDF groups was significantly increased, among which *Norank_F_Muribaculaceae, Bacteroides, Alloprevotella, Megasphaera, Rikenellaceae_RC9_gut_group,* and *Lactobacillus* were relatively rich in each group. The proportion of intestinal flora of *Norank_F_Muribaculaceae* was negatively correlated with the dose of unmodified, while it was positively related to the dose of enzymatic-modified dietary fibre. This suggests that unmodified inhibited the growth of *Norank_F_Muribaculaceae*, while enzymatic-modified dietary fibre promoted the growth of *Norank_F_Muribaculaceae*. In addition, administration of dietary fibre inhibited the growth of *Alloprevotella*. The inhibitory activity of enzymatic-modified dietary fibre was significantly higher than that of unmodified dietary fibre.

3.5. The effects of administration of potato residue dietary fibre on the production of SCFAs of mice

SCFAs refer to organic FAs with less than 6 carbon atoms in the carbon chain. Dietary fibre can be metabolized by anaerobic bacteria fermentation in the intestinal cavity (Lewis et al., 2019). The fermentability of dietary fibre in the intestinal tract of rat can be determined by detecting the content of SCFAs in the faeces of mice. Fig. 5 depicts the

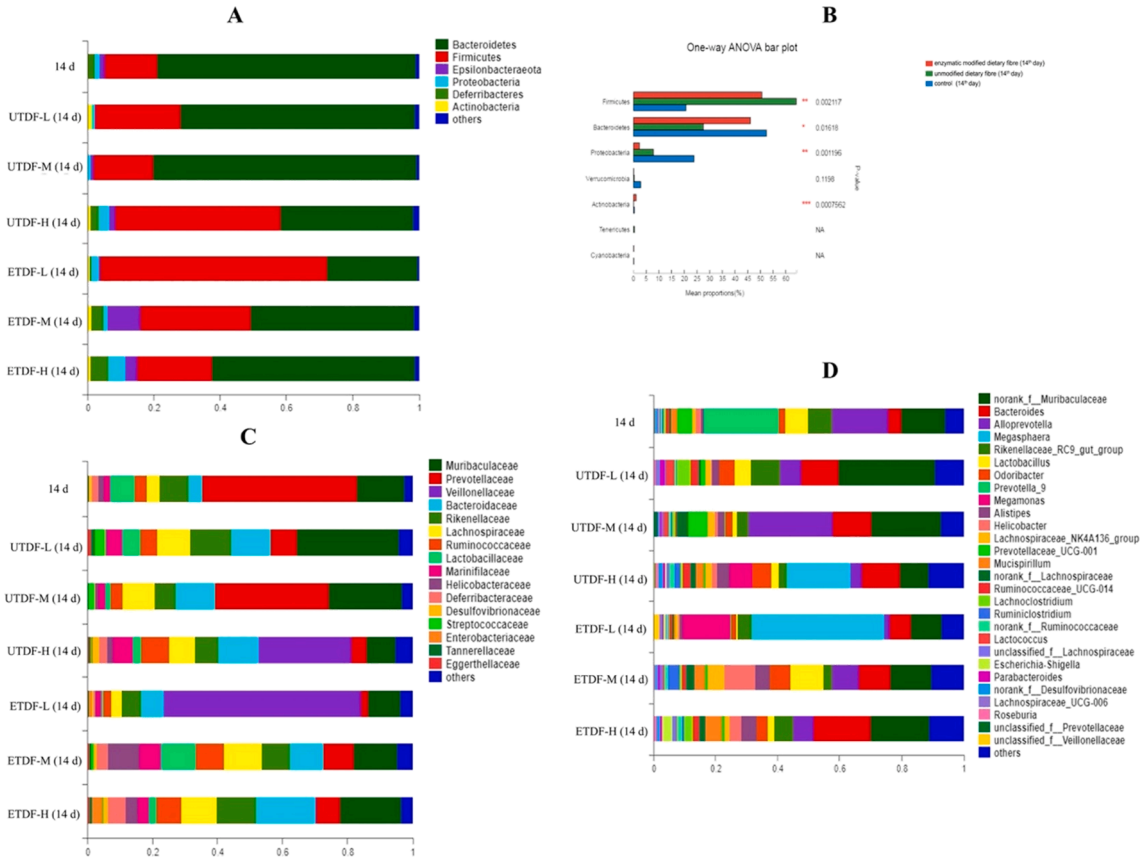

Fig. 4. . The composition of the intestinal flora in mice at phylum (A), family (B), and genus (D) levels; The proportions of Firmicutes and Bacteroidetes of control, unmodified, and enzymatic-modified dietary fibre groups (C).

changes in the contents of acetic acid (a), propionic acid (b), n-butyrate (c), isobutyrate (d), n-valeric acid (e) and isovaleric acid (f) in faeces of mice treated with unmodified and enzymatic-modified dietary fibre, respectively, at low, medium, and high doses for 14 days. Acetic acid has been shown to inhibit the growth of tumour cells (Marques et al., 2013). In this study, acetic acid accounted for the highest proportion of SCFAs detected in faeces of mice, followed by propionic acid, n-butyric acid, and isobutyric acid. The content of isovaleric acid was the lowest in the faeces of mice. According to the Fig. 5, there was no significant difference in the content of each type of SCFA among all groups on day 0. It has been reported that butyric acid can be used as an important energy source of epithelial cells to maintain the stability of intestine and prevent colorectal cancers (Lamichhane et al., 2020) while valeric acid can effectively inhibit the growth and reproduction of harmful clostridium (L. Su et al., 2020). Herein, the contents of acetic acid, n-butyric acid, isobutyric acid, n-valeric acid and isovaleric acid in faeces of mice were significantly increased by administration of unmodified- or enzymatic-modified dietary fibre at different doses. The contents of acetic acid, n-butyric acid, isobutyric acid, n-valeric acid and isovaleric acid in enzymatic-modified dietary fibre-treated group were significantly higher than those in unmodified dietary fibre-treated group ($p < 0.05$). After administration of the high dose of enzymatic-modified dietary fibre for 14 days, the contents of acetic acid (2758.02 μg/g), n-butyric acid (923.79 μg/g), n-valeric acid (115.16 μg/g), and valeric acid (102.33 μg/g) were significantly higher than those administration of the corresponding unmodified dietary fibre, with the values of acetic acid, n-butyric acid, n-valeric acid, and valeric acid 2485.35, 787.76, 84.57, and 76.13 μg/g, respectively ($p < 0.05$). Previous study has already reported that anaerobic fermentation of fibres is the largest source of SCFAs. SCFA concentrations and/or SCFA producing-bacteria can be strengthened by fibre-rich diets. Hence, it could be concluded that dietary fibre might be the main contribution for the increasing contents of SCFAs. In addition, ETDF promoted the production of acetic acid, n-butyric acid, isobutyric acid, n-valeric acid and isovaleric acid in the faeces of mice, and this promotion effect was stronger than that of UTDF. Gao et al. (2018) has reported that propionic acid participates in the regulation of immune pathways and may have negative influence on the immune response. Herein, administration of dietary fibre considerably reduced the content of propionic acid in the faeces of mice. After administration of high dose of enzymatic-modified dietary fibre for 14 days, the propionic acid content (428.03 μg/g) in faeces of mice was remarkably lower than that in the corresponding unmodified dietary fibre-treated group (448.31 μg/g, $p < 0.05$). Therefore, treatment with the potato residue dietary fibre exhibited the inhibitory effect on the production of propionic acid in the faeces of mice. Modification of cellulase and xylanase enzymes for the potato residue dietary fibre strengthened this inhibitory effect on the production of propionic acid.

Fig. 6 displays the heat map of correlation analysis between microbial community distribution and SCFAs. X and Y axis show the classification of SCFAs and intestinal flora at the genus level, respectively. Acetic acid was significantly positively correlated with *Lactobacillus* ($p < 0.05$), and negatively correlated with *Ruminiclostridium_5, Ruminiclostridium, Unclassified_f_veillonellaceae, Megasphaera*, and with *Megamonas*. Propionic acid is shown to be positively correlated with *Norank_Muribaculaceae, Parasutterella, Coriobacteriaceae_UCG-002, and Muribaculum*, positively relative to *Parasutterella* extremely ($0.001 < p < 0.01$), while it is negatively correlated with *Candidatus_Saccharimonas,* and *Ruminiclostridium_5*. There was a significant negative correlation between n-butyric acid and Bacteroides, whilst significant positive correlations between isobutyric acid and *Roseburia, Norank_F_Norank_O_Mollicutes_RF39*, and with *[Eubacterium]_Fissicatena_group* were all observed. Isovaleric acid is positively correlated with

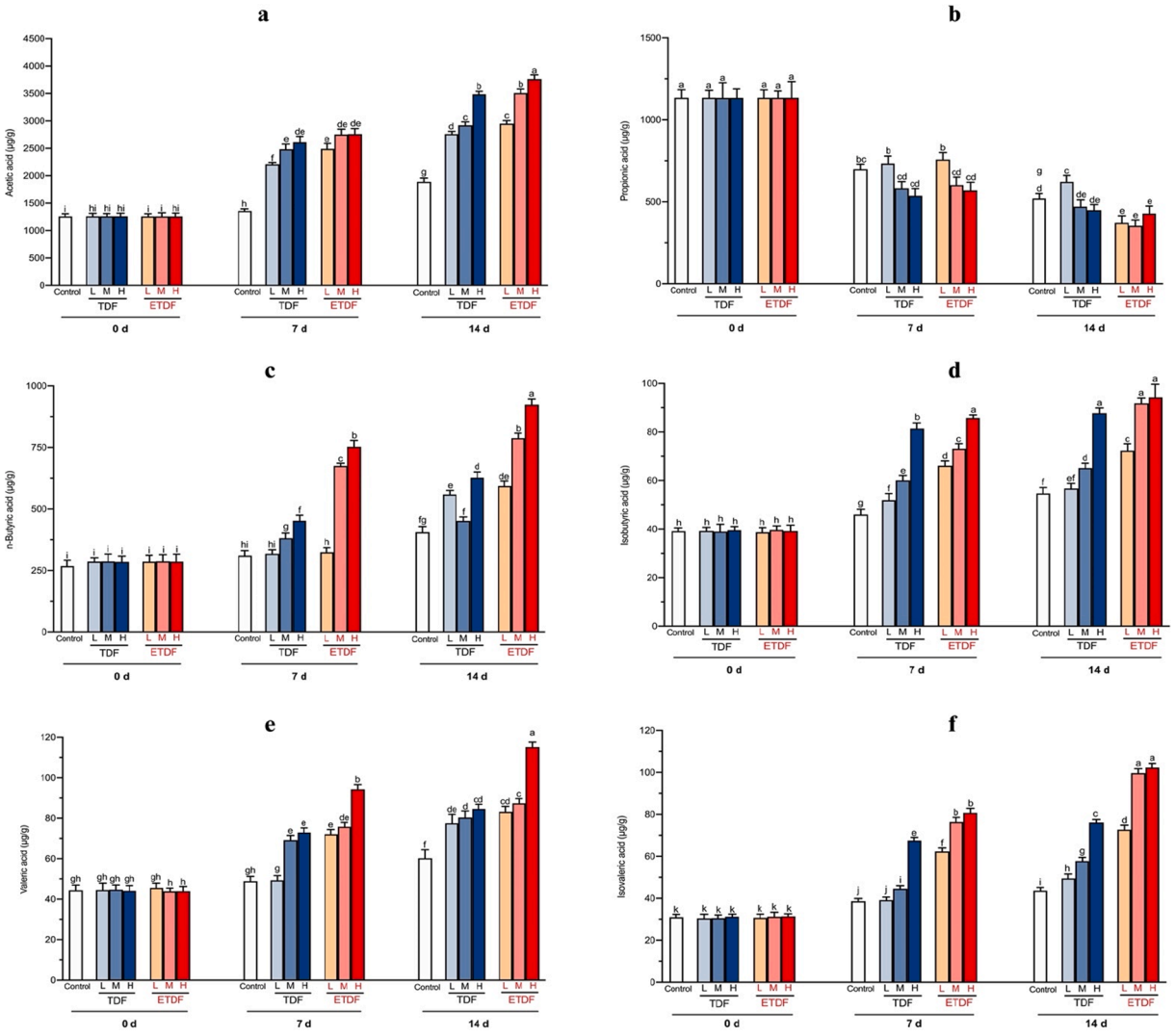

Fig. 5. . The contents of acetic acid (a), propionic acid (b), n-butyrate (c), isobutyrate (d), n-valeric acid (e) and isovaleric acid (f) in faeces of mice treated with unmodified and enzymatic-modified dietary fibre, respectively, at low, medium, and high doses for 14 days.

[Eubacteriun]_Fissicatena_group and *Ruminococcaceae_UCG-014,* and with *Norank_F_Norank_O_Mollicutes_RF39*. However, there was no obvious relationship between

n-valeric acid and intestinal microflora. According to previous studies that SCFA were produced by the gut microbiota by fermenting fiber and indigestible polysaccharides (Barczynska et al., 2017). Barczynska et al. (2017) have reported that the consumption of potato dextrin caused a substantial increase in total SCFA as well as in some individual acids in the distal intestine of mice.

Dietary fibre exhibits a fantastic fermentation property in the intestinal tract, which in turn, regulates the composition of microorganisms (Williams, Grant, Gidley, & Mikkelsen, 2017). Holscher (2017) reported that potato dietary fibre with beneficial bio-characteristics promoted the growth of *Bacteroides* strains and inhibited the growth of the *Firmicutes* strains, thus effectively preventing the risk of obesity. Under the synergism of gut microbes, dietary fibre is fermented by microflora in large intestine. As the dietary fibre fermentation metabolic products, SCFAs affect intestinal pH values, change the distribution of the intestinal flora and the species richness, increase the beneficial bacteria, and restrain the harmful bacteria (Parada Venegas et al., 2019). Those unfermented dietary fibres can be excreted in the form of faeces with the fermented residues due to their water holding capacity (WHC) and swelling power (SP) in the intestinal tract, consequently maintaining the health of the intestinal tract and human body and reducing the occurrence of disease (Williams, Mikkelsen, Flanagan, & Gidley, 2019). This is also consistent with the findings of our previous study, revealing that modification of enzymes for the dietary fibre increased its WHC and SP, thus promoting the production of SCFAs and probiotics.

4. Conclusion

In this study, the dietary fibres (unmodified and modified by cellulase and xylanase) extracted from potato residue were used. The mice were administrated with these dietary fibres to evaluate and compare the effects of the unmodified and enzymatic-modified potato dietary fibres on the distribution of intestinal microbiotas and the production of SCFAs. The findings revealed that administration of both unmodified and enzymatic-modified dietary fibres extracted from potato residue could promote the production of acetic acid, n-butyric acid, isobutyric acid, valeric acid, and *iso*-valeric acid, while inhibited the production of propionic acid. In addition, the dietary fibre extracted from potato residue significantly improved the number and diversity of intestinal microflora of mice, in particular the increased ratio of *Bacteroidetes* to *Firmicutes*. Cellulase- and xylanase-modified potato residue dietary fibre exhibited significant effects on SCFAs and intestinal microbiotas than unmodified dietary fibre, due mainly to the increased soluble

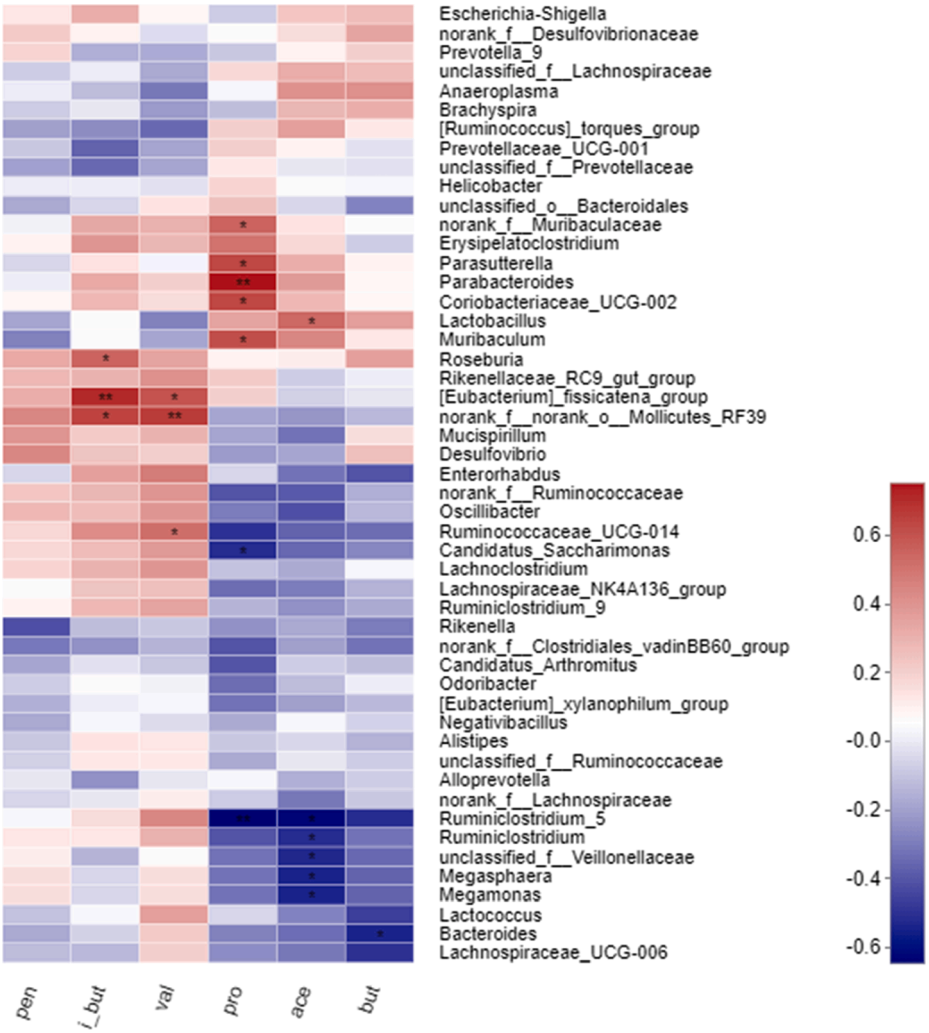

Fig. 6. . Heatmap chart of correlation between intestinal bacterial community and short chain fatty acids.

components, and stronger physicochemical and functional characteristics of the enzymatic-modified dietary fibre, such as water holding capacity, swelling capacity, and glucose absorption capacity. Taken together, it is certainly clear that dietary fibres extracted from the potato residue have the potential to selectively alter the distribution of gut microbiota of mice and could therefore act as a therapeutic agent to treat dysbiosis in the intestinal microbiota or prevent the risk of obesity.

CRediT authorship contribution statement

Qianyun Ma: Conceptualization, Methodology, Investigation, Software, Validation, Resources, Writing - review & editing, Project administration. **Wenxiu Wang:** Conceptualization, Methodology, Investigation, Validation, Resources, Writing - review & editing. **Ziye Ma:** Validation, Formal analysis, Data curation. **Yaqiong Liu:** Investigation, Writing - review & editing. **Jianlou Mu:** Software, Validation. **Jie Wang:** Methodology, Resources. **Letitia Stipkovits:** Methodology, Investigation. **Gang Wu:** Writing - review & editing. **Jianfeng Sun:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Xiaodan Hui:** Conceptualization, Methodology, Investigation, Writing - original draft.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104606.

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